BIOLOGICAL INCISING: NATURE'S WAY OF IMPROVING THE TREATABILITY OF SPRUCE AND PINE.

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Abstract

Canadian wood species such as spruce and pine are difficult to treat with wood preservatives or other wood enhancing formulations due to a thin sapwood band and refractory heartwood. Biological incising with *Dichomitus squalens* was developed in Austria in the 1990s to increase the permeability of European spruce. It was considered unlikely that industrial use of a European white-rot fungus would be acceptable in Canada. FPInnovations therefore screened a range of Canadian isolates of various white-rot fungus species and found one isolate of *D. squalens* from white spruce that greatly increased permeability in spruce. The objective of the current study was to determine if similar results could be achieved on commercial sized wood under non-sterile conditions more similar to an industrial setting. Short lumber samples were incubated in plastic totes with fungal inoculum. Two isolates were tested with two decontamination methods and two time frames (4 and 6 weeks). Through treatment of spruce samples (19 mm penetration) with 1.7% ACQ was achieved after six weeks incubation with a spruce isolate. In matched samples treated with MCA, a minimum of 10 mm penetration was achieved in 90% of the samples. Strength loss in some individual samples was higher than adjustment factors for conventional incising (over 25%) suggesting that incubation time may need to be shortened. Preservative penetration was more variable in pine but permeability was increased; 60% of the samples reached a minimum penetration of 5 mm. Incubation time and conditions need to be adjusted to achieve more consistent results. Future work will focus on determining incubation conditions that allow penetration requirements in Canadian standards to be met with acceptable strength loss.

1. Introduction

Canadian wood species such as spruce and pine are difficult to treat with wood preservatives or other wood enhancing formulations due to a thin sapwood band and refractory heartwood. Since these species are not naturally durable, they can be prone to fungal decay. The difficulty with preservative treatment of these species limits their use under certain conditions, such as ground contact applications, and can reduce the competitiveness for these species in the marketplace.

Seifert and Morris (1987) discussed the potential of biological incising in a strategy document for Forintek's (now FPInnovations) biological control initiative. Previous work in this field had focused on improving the permeability of sapwood (Bergman 1984) but for Canadian species there was a need to focus on fungi that would affect heartwood. In 1988, limited exploratory work was done at Forintek using *Stereum sanguinolentum*, (Albertini & Schwein.:Fr.) Fr., a red-heart fungus, on spruce heartwood with limited success (unpublished data). Red-heart is known to increase permeability but at the early stages of colonization it has little effect on strength (Roff and Whittaker 1963). This work was abandoned due to the need to focus efforts on improved

mechanical incising at that time.

In 1998, Rosner *et al*. screened two *Trichoderma* species (moulds) and two white-rot basidiomycetes, *Dichomitus squalens* P.Karst and *Phanerochaete chrysosporium* Burdsall on Norway spruce logs. Only *D. squalens* penetrated the heartwood. Creosote uptake (based on weight) increased the most in logs that had been exposed to *D. squalens* for three weeks. Average creosote loadings were 203.8 kg/m^3, over twice the uptake of controls. However, some reduction was observed in Modulus of Rupture (MOR) and Modulus of Elasticity (MOE) (Rosner *et al*. 1998). In 2000, patents were taken out on this process (Messner *et al.* 2000) and some collaborative work was initiated between the University of Vienna, Oregon State University and Forintek. Forintek provided spruce lumber to Oregon State and the University of Vienna provided the inoculum. This experiment was unsuccessful due to problems with growth of the liquid inoculum. Other priorities combined to prevent follow up work.

In Switzerland more recent studies have focused on using *Physisporinus vitreus* (Pers.) P. Karst to improve permeability in Norway spruce and white fir (Schwarze *et al*. 2006, Schubert *et al*. 2010). Heartwood samples of Norway spruce and white fir exposed to two isolates of *P. vitreus* showed an increase in water uptake after 6 weeks of incubation with average uptakes of 300-400 kg/ m^3 in Norway spruce and 400- 680 kg/ m^3 in white fir (Schwarze *et al*. 2006). No significant strength loss was found in Norway spruce; however, there was some strength loss in white fir (Schwarze *et al*. 2006). Wan *et al*. (2006) used *Gliocladium roseum* Bainier, *Ophiostoma piceae* (Munch) H. & P. Sydow and *Trametes versicolor* (L.) Lloyd to biologically incise selected hardwoods prior to impregnation with chemicals to improve hardness.

Recently the patents on the original Austrian work (Messner *et al*. 2000) lapsed due to non payment of fees. It was considered unlikely that industrial use of a European white-rot fungus would be acceptable in Canada. In 2009, FPInnovations screened selected Canadian isolates of white-rot and red-heart on white spruce (*Picea glauca* Moench, Voss) and jack pine (*Pinus banksiana* Lamb.) heartwood (Dale *et al*. 2010). An isolate of *D. squalens* from white spruce showed very promising results in pure culture on spruce samples. Preservative uptake after six weeks of fungal exposure averaged about 690 kg/m^3, which was eight times higher than uptake in the control blocks. Preservative penetration was achieved through the entire 19 mm samples, whereas penetration averaged one millimetre in unexposed control blocks. No reduction in stiffness was found in these samples based on perpendicular-to-grain tangential compression testing. A second isolate isolated from pine also showed some promise. *D. squalens* is a white rot basidiomycete commonly found causing red-heart of Ponderosa pine (Andrews 1971). Incipient decay of wood is characterised by the red discolouration with no obvious changes in structure. As decay progresses, white pocket rot starts to appear (Andrews 1971).

Based on these results, a larger scale test was set up to determine whether enhanced preservative penetration with minimal strength loss could be achieved in lumber exposed to the fungus in an unsterile setting. Treatment was primarily with Alkaline copper quat (ACQ), but at the request of Timber Specialties Co., and financially supported by them, selected matched samples were also treated with micronized copper azole (MCA).

2. Materials and Methods

2.1 Sample Preparation

Freshly cut western white spruce logs from near Merritt, BC (an interspecies mix and hybrids of *Picea engelmannii* Parry and *Picea glauca* Moench, Voss) were cut into boards approximately 5 cm thick by 10 cm wide by 500 cm long. Twenty boards were selected from two logs from two different trees avoiding boards containing only sapwood, pith, bark or wane, and obvious defects. Boards were cut into twelve 400 mm samples and divided into four treatment groups with three end-matched samples per group. The end-matched samples were assigned to incubation times in the order of 4, 0, 6 weeks so that each test sample was adjacent to the control (0 weeks). This process was repeated ten times to produce ten replicates per treatment, per time frame.

Twelve logs (some of which may have come from the same tree) of green lodgepole pine (*Pinus contorta var. latifolia* Engelm.) unaffected by mountain pine beetle, obtained from near Edson, Alberta, were cut into rough green 5 cm thick by 10 cm wide by 150 cm boards, also avoiding bark, wane, and obvious defects. Boards were cut into three 400 mm samples and assigned to a treatment group. The three end-matched samples were assigned to incubation times in the order of 4, 0, 6 weeks so that each test sample was adjacent to the control (0 weeks). The ends of pine and spruce samples were sealed with two coats of two-part epoxy-resin (Intergard 740, International Paint, Houston, TX, USA).

There were four different treatment groups testing two decontamination methods and two moisture levels. Group 1 was kiln heated in a steam atmosphere to a core temperature of 70°C to decontaminate and left at green moisture content, Group 2 was pressure treated with water (2 hrs at 150 psi) to saturate any sapwood present and then kiln heated to 70 °C to decontaminate, Group 3 was dipped in a 10 ppm Benomyl solution intended to kill mould fungi without affecting subsequent colonization by basidiomycetes and left at green moisture content, and Group 4 was pressure treated with water (2 hrs at 150 psi) to saturate any sapwood present and then dipped in a 10 ppm Benomyl solution. The sapwood was saturated in half of the samples to attempt to force the fungus to preferentially colonize the heartwood rather than the sapwood.

2.2 Inoculum Preparation

Two isolates of *D. squalens* were selected, one isolated from spruce and one isolated from pine. Details of inoculum preparation are being kept confidential.

2.3 Sample Exposure

Pine and spruce samples from the same treatment were arranged in 18 gallon Roughneck Totes (Rubbermaid). Samples were layered, alternating spruce and pine, separated by 1 cm wide strips of corrugated plastic sheets (Coroplast, Granby, Quebec) and inoculum was applied. Non-experimental spruce 1x4 boards were placed on the top and bottom of the stack, and the stack was raised on a 1.5” ABS pipe above water to keep the humidity high. The lids were placed upside down on the totes (to reduce the formation of water droplets immediately above the samples) and sealed with duct tape. This arrangement was repeated for each treatment and fungal isolate. Bins

were incubated at a relative humidity of between 80-85% and 20°C for 2 weeks (due to a fault in temperature control) and then raised to the intended temperature of 25°C for an additional 2 or 4 weeks for a total incubation time of 4 weeks and 6 weeks. Time zero samples (no fungal exposure) were frozen until the end of the incubation time. After incubation, all steam-decontaminated and time zero samples were kiln heated to a core temperature of 80°C to kill the fungus and denature fungal enzymes (Périé *et al*. 1998 found that laccase activity is reduced by 90% at a temperature of 75°C for 10 minutes) and then dried between 60 - 84°C for 44 hours until they reached between 12% and 20% moisture content. Moisture content was measured with a capacitance type moisture meter set to lodgepole pine (Wagner Electronics, Rogue River, Oregon, USA). Benomyl treated samples were not processed further due to low colonization and high contamination.

2.4 Sample Preparation for Treatment and Strength Testing

Steam decontaminated samples exposed for zero and six weeks as well as steam decontaminated, green four week samples exposed to the spruce isolate were cut into four subsamples (A to D) for preservative treatment, strength and durability testing (Figure 1).

The dashed line shown in Figure 1 indicates initial rough green dimension, the solid lines indicate final dry planed dimension, and the two curves indicate heart/sap boundaries. Sample A was subjected to a static bending test as described below with the arrow labeled face in tension, sample B was intended for a toughness test with the arrowed labeled face impacted (not initiated due to funding cuts), sample C was cross cut into two 200mm long samples, for preservative treatment and penetration measurements. Sample D was intended for treatment and durability testing (not initiated due to funding cuts).

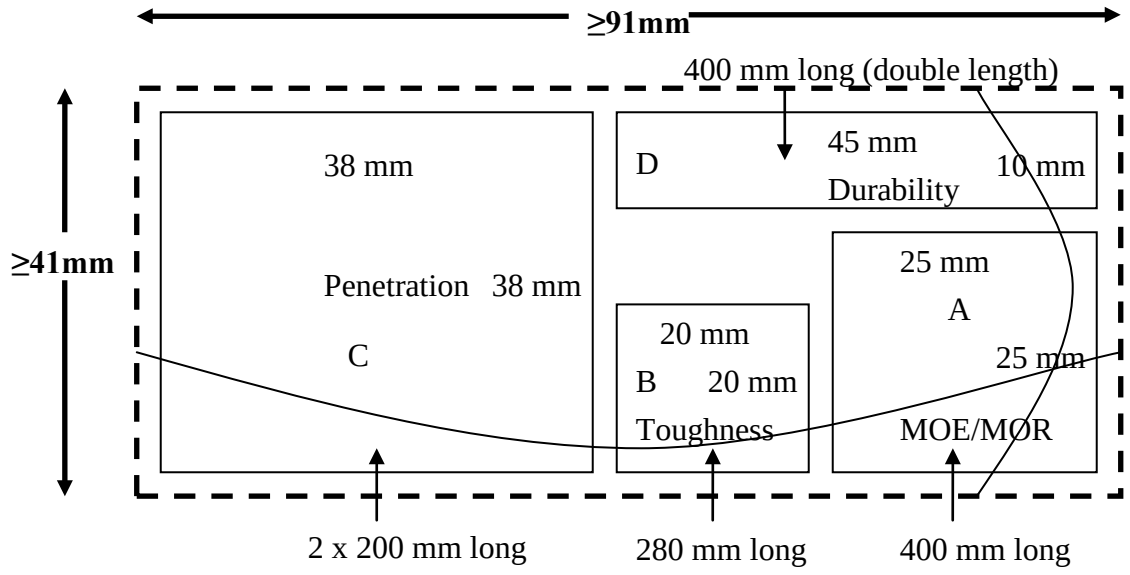

Figure 1 Cutting pattern for sub-samples from bio-incised 5 cm by 10 cm boards (cross section view).

2.5 Preservative Treatment

Each of the 38 x 38 x 200 mm samples (sub-sample C shown in Figure 1) from each of 10 boards were sealed with two coats of epoxy resin (Intergard 740, International Paint, Houston, TX, USA) on the ends and along the unexposed face (the surface adjacent to sub-sample D and B) to simulate half a board. Samples were weighed and one was pressure treated with 1.7% ACQ using the following conditions: 30 minute vacuum at 28 in Hg, 2 hour pressure treatment at 150 psi, and 15 minutes vacuum at 28 in Hg. With the 6 week exposure, the second half was treated with 0.9% MCA using the same process conditions. After treatment, samples were reweighed and preservative uptake was calculated for each sample, and averaged for each treatment group. The steamed, green, four week samples exposed to the spruce isolate were pressure treated with 2.0% ACQ using the same conditions.

Steamed, green pine and spruce exposed to the spruce isolate and the respective controls were cross cut into three equal segments and the cross sections were sprayed with the copper indicator, chrome azurol S, to indicate the depth of penetration of the preservative. Preservative penetration was measured at the heartwood face and one edge of each sample at the three cross cut positions. Average penetration and the percentage of samples with equal to or greater than 5 mm and 10 mm penetration was calculated for the heartwood face and edge face for each group of samples.

2.6 Strength Testing

The specimens were conditioned at 20°C and 65% RH prior to testing. Bending strength testing (Figure 5) was done on 10 replicates each of green spruce that had been exposed for four weeks green spruce and pine exposed to the spruce isolate for six weeks, and end-matched unexposed samples. Samples with large knots were not tested. The 25 x 25 x 400 mm subsample (Figure 1-A) was tested in accordance with section 8 of the standard test method D143 (ASTM 2010). The Modulus of elasticity (MOE) and Modulus of Rupture (MOR) were calculated. The relative density of all six week and control samples was determined using ASTM D2395, Method B (Section 10.2.5) with no surface treatment.

3. Results and Discussion

3.1 Colonization after Fungal Exposure

The extent of colonization was moderate in the four week samples but was high in all of the six week steam treatments (Figure 1 D&E). Contamination was low in all steam treatments, except one four week set. Overall, steam treatment appeared to be an effective method of reducing contamination to allow the growth of *D. squalens* with little to no competition. The Benomyl treatment technique did not adequately inhibit the growth of contaminants, mainly a *Trichoderma* sp. Based on these results the Benomyl treated samples were dropped from the study; steamed six week samples and steam, green spruce isolate four week samples were pressure treated and analyzed for uptake (see below).

3.2 Preservative Uptake and Penetration

There was a substantial increase in preservative uptake in the green spruce samples (those that had not been saturated with water) exposed to the spruce isolate for six weeks compared to the controls (0 weeks exposure, Table 1). Average ACQ uptake (based on weight) was 526 kg/m^3 (32.8 lbs/ft^3) which was over seven times higher than the average for the controls (zero weeks), which was 73 kg/m^3 (4.5 lbs/ft^3). Results were similar for the MCA treated spruce. Uptake in the biologically incised samples (544 kg/m^3 or 34.0 lbs/ft^3) was over four and a half times higher than in the controls (117 kg/m^3 or 7.3 lbs/ft^3). The controls in the MCA treated samples took up more solution than expected, but penetration was not higher in the MCA treated samples compared to the ACQ treated samples (Table 3, Figures 2 and 3). Based on these results, the four week, steamed spruce samples exposed to the spruce isolate were also treated. Uptake increased over four and a half times to 347 kg/m^3 (21.6 lbs/ft^3).

There was also an increase in uptake in the biologically incised, green pine samples exposed to the spruce isolate; however it was not as high as in the spruce, probably due to pinosylvins in pine heartwood that this spruce isolate is not adapted to. Uptake of ACQ increased from 157 kg/m^3 (9.8 lbs/ft^3) in the controls to 309 kg/m^3 (19.3 lbs/ft^3) in the bio-incised samples. The difference was not as drastic for the MCA-treated pine, since the control samples took up more chemical than expected (Table 1). It is unusual to have higher uptake of MCA in un-incised samples and the reason for higher uptakes in the pine control samples is unknown at this time.

The biological incising was not as effective using the pine strain of *D. squalens* on either the spruce or pine (Table 1) presumably because the strain is slower growing on a wood substrate. The largest increase in uptake was in the green spruce bio-incised samples which showed a 3-fold increase in uptake. Pressure treating the wood with water did not improve penetration into the heartwood suggesting that the increase in moisture did not provide a good environment for fungal growth. This fungus may be adapted to lower wood moisture suggested by its occurrence in exposed and dry habitats such as in the dry regions of South Central Africa (Masuka and Ryvarden 1999) and in logged and burned sites (Junninen *et al*. 2008).

Table 1 Preservative uptake in biologically -incised spruce and pine versus control samples

Moisture Condition	Fungal Isolate	Average Uptake* (kg/m^3)				
		ACQ			MCA	
		0 weeks exposure	4 weeks exposure[a]	6 weeks exposure	0 weeks exposure	6 weeks exposure
		Spruce				
Green	spruce	73 (34)	347 (103)	526 (125)	117 (50)	544 (105)
Saturated	spruce	105 (61)	N/T	153 (93)	135 (40)	158 (109)
Green	pine	96 (39)	N/T	275 (206)	105 (32)	318 (190)
Saturated	pine	118 (46)	N/T	297 (196)	142 (36)	345 (184)
		Pine				
Green	spruce	157 (205)	N/T	309 (137)	254 (143)	380 (119)
Saturated	spruce	188 (135)	N/T	191 (154)	247 (98)	288 (139)
Green	pine	108 (141)	N/T	277 (141)	172 (132)	321 (156)
Saturated	pine	183 (160)	N/T	217 (108)	241 (143)	283 (119)

* Standard deviation in brackets, sample size = 10 for each treatment and exposure
[a] Only 4 week samples treated for the steam sterilized, green spruce with isolate the spruce isolate. Others not tested (N/T)

All six week biologically incised spruce blocks except one were treated all the way through (19 mm) with ACQ (Figure 2). In the four-week samples, average penetration through the heartwood face was 7 mm and 9 mm through the edge (Table 2). Seventy percent of the samples reached a minimum of 5 mm on the heartwood face, and 80% reached 5 mm through the edge. The average penetration for the control spruce samples was 1 mm at the heartwood face, and 2 mm on the edge; only one sample reached greater than 5 mm penetration at one point along the edge.

Similar results were found in the biologically incised spruce treated with MCA (Figure 3). Average penetration was 16 mm at the heartwood face, and 16 mm at the edge. A minimum of 10 mm penetration was reached in 90% of the samples on the heartwood face and 80% of the samples on the edge. Average penetration in controls was 1 mm, and no samples reached 5 or 10 mm.

Penetration was also increased in the biologically incised pine treated with ACQ (Table 2, Figure 2); however, not as much as in the spruce samples. Incubation time may need to be longer to reduce the variability and to achieve through treatment. Results were more pronounced on the heartwood face where average penetration was increased from 1 mm in the controls to 8 mm in the bio-incised samples. On the edge face, penetration increased from 6 mm in the controls to 11 mm in the biologically incised samples. Sixty percent of the biologically incised samples reached a minimum of 5 mm penetration on the heartwood face, and 70 % for the edge face compared to 0 % and 30% on the heartwood face and edge face of the controls.

For pine treated with MCA, results were more variable (Table 2, Figure 3), particularly in the

controls which ranged from 0 mm to 19 mm with a mean of 11 mm on the heartwood face and 12 mm on the edge due to the presence of sapwood in several samples. There was a small increase in the biologically incised samples with a range from 3 mm to 19 mm and a mean of 13 mm on the heartwood face, and 16 mm on the edge. Seventy percent of the controls achieved a minimum of 5 mm penetration which increased to 80% penetration on the heartwood face with biological incising and 100 % on the edge.

Table 2 Average penetration of ACQ and MCA in biologically incised spruce and pine and in control samples and percent of samples reaching minimum treatment thresholds.

	Exposure Time	**1.7% ACQ**							
		Heartwood Penetration (mm)				**Edge Penetration (mm)**			
		Mean	SD	% ≥ 5mm	% ≥ 10mm	Mean	SD	% ≥ 5mm	% ≥ 10mm
Spruce	0 weeks	1	1	0	0	2	1	10	0
Spruce*	4 weeks	7	4	70	30	9	5	80	50
Spruce	6weeks	19	1	100	100	18	5	90	90
Pine	0 weeks	1	1	0	0	6	7	30	30
Pine	6weeks	8	7	60	30	11	7	70	60
		0.9% MCA							
Spruce	0 weeks	1	0	0	0	1	0	0	0
Spruce	6weeks	16	4	100	90	16	6	90	80
Pine	0 weeks	11	8	70	60	12	8	70	60
Pine	6weeks	13	7	80	50	16	6	100	70

* treated later with 2.0% ACQ
Sample size = 10 for each treatment
Only green samples exposed to the spruce isolate were measured for penetration

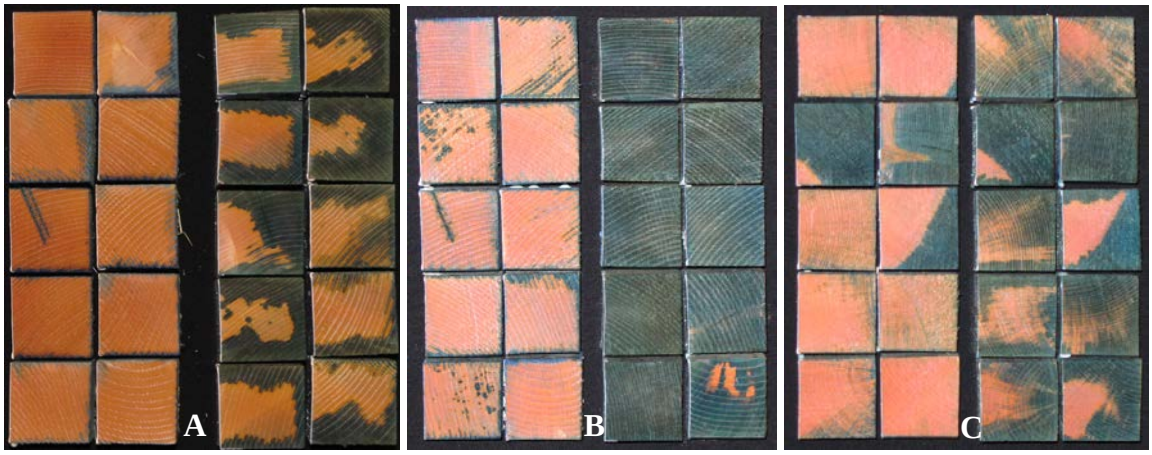

Figure 2 ACQ uptake in 4 week exposed spruce (A), 6 week exposed spruce (B) and 6 week exposed pine (C) with controls (left) versus biologically-incised samples exposed to* D. squalens *(right). Blue coloration is from the copper indicator, chrome azurol S and represents the treated area

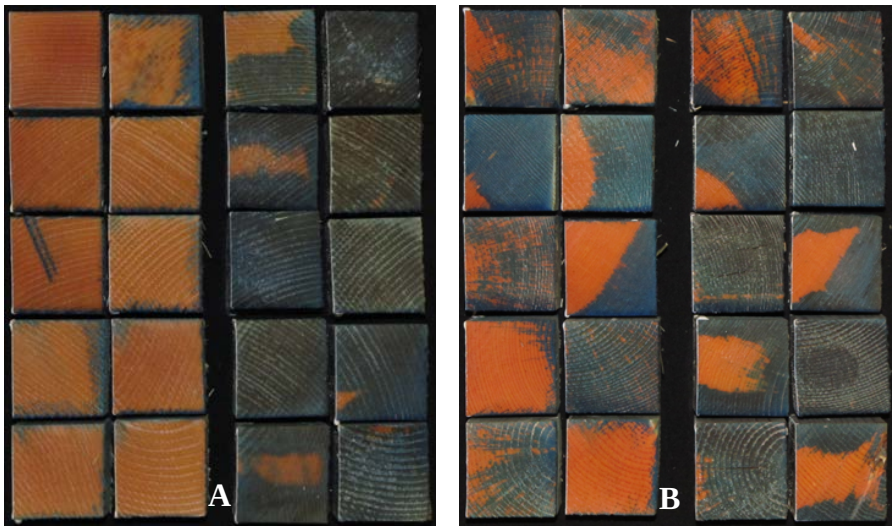

Figure 3 MCA (0.9%) uptake in spruce (A) and pine (B) with controls (left) versus biologically-incised samples exposed to* D. squalens *for six weeks (right). Blue coloration is from the copper indicator, chrome azurol S and represents the treated area

3.3 Strength Loss

Due to the small number of logs sampled, the spruce and lodgepole pine may not be representative of the species. Therefore the results apply only to the wood samples tested.

In spruce, there was an average reduction of 4% in MOR after four weeks of biological incising and a 21% reduction in MOR after six weeks for the biologically incised samples compared to the controls (Figure 4). Reduction in MOR ranged from 0 to 15% in four week samples and 9% to 37% in six weeks samples. Reduction in MOE ranged up to 18 % with an average loss of 8% in

the four week samples, and up to 13% with an average loss of 5% in the six week samples.

In the pine after six weeks incubation, there was an average reduction in MOR of 12% with a maximum loss of 27% in one sample. Average reduction in MOE was 8% with a maximum of 23%. Four week samples were not tested for pine.

In the biologically incised spruce, 100% of the six week samples had a tension failure, 0% of the four week samples had a tension failure (all were compression failures), and 20% of the controls had a tension failure (Figure 5). Eighty-three percent of the biologically incised pine had a tension failure after six weeks while 0% had a tension failure in the controls.

The average relative density was 0.392 (SD = 0.016) in the spruce controls, and 0.379 (SD = 0.011) in the six week spruce samples. The decrease in density in the spruce samples could be due to decay initiation as a result of the length of the incubation period. In the pine, the average relative density was 0.448 (SD = 0.057) in the controls and 0.457 (0.034) in the six week samples.

The loss in strength is higher than that typically found in mechanically incised wood. Lam and Morris (1991) found losses in MOE as a result of mechanical incising ranging from 5% - 15% and losses in static strength properties ranging from 10% - 30%. Winandy and Morrell (1998) found reductions of 0% - 10% in MOE and 15% – 25% in MOR in mechanically incised Douglas-fir, Hem-Fir, and Spruce-Pine-Fir 2x4 lumber. The Canadian code for engineering design in wood, CSA O86 (CSA, 2009) allows for a loss of 10% in MOE and 25% in other properties (including MOR) for preservative-treated incised lumber less than 89 mm thick. Decreasing incubation time during the biological incising process may reduce the strength loss to within the range assumed by CSA O86 for mechanical incising, while still meeting standards for preservative penetration. The strength losses observed in this study are for clear wood. In full-size lumber, the actual strength loss may be lower because of the presence of more dominant strength-reducing characteristics such as knots.

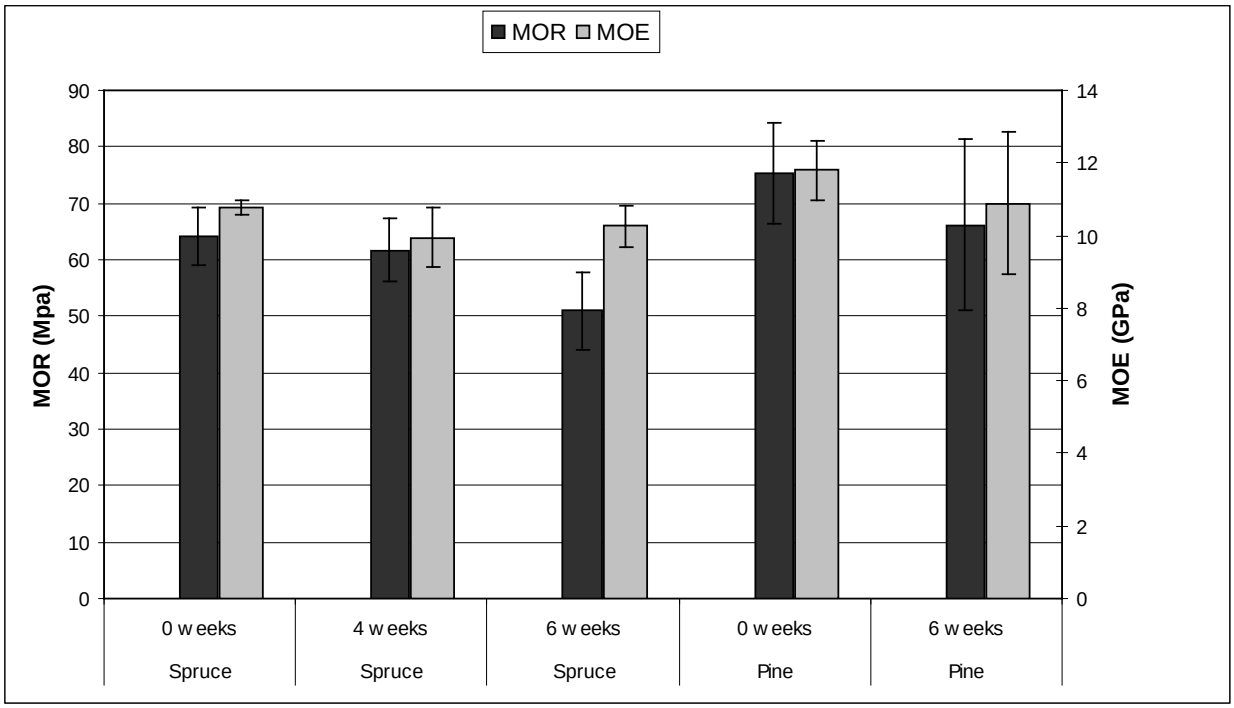

Figure 4 MOE and MOR results from the strength testing of spruce and pine samples biologically incised for with D. squalens versus control samples with no biological incising
Spruce sample size = eight; Pine sample size = 5. Error bars represent standard deviation.

Figure 5 Bending strength test (A), compression failure (B) and tension failure (C)

Figure 6 shows the relationship between strength loss expressed as MOR and ACQ uptake. A moderate correlation was found between the two variables with an R^2 value of 0.43. This suggests that aiming for a solution uptake of approximately 400 to 450 kg/m^3 could result in strength losses within the acceptable range of 25% as indicated by the CSA standards. The difficulty with making such a prediction is that it does not take into account the variability in the data; however, it could aid in setting targets for future studies aimed at finding an acceptable level of both penetration and strength loss.

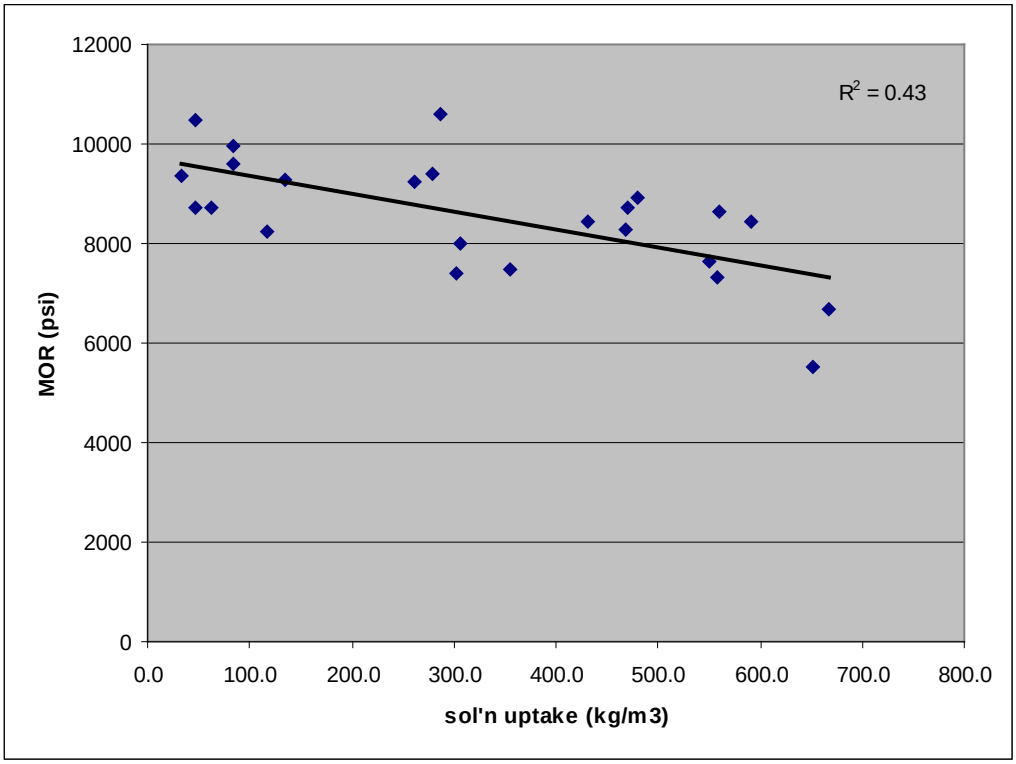

Figure 6 Correlation between MOR and solution uptake for spruce samples including control, 4 week and 6 week biologically incised samples

4. Conclusions

Biological incising was successful using an isolate of *D. squalens* from spruce on green, steam-decontaminated 2x4 spruce and pine samples. In spruce samples, through treatment was achieved using 1.7% ACQ in nine of ten samples. Near through treatment was achieved in seven of ten samples using 0.9% MCA. Strength loss in some samples was higher than that typically caused by mechanical incising, ranging as high as 37% reduction in MOR and 13% reduction in MOE in spruce samples and 27% reduction in MOR and 23% in MOE for pine samples.

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